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INTERACTION OF LEUKOTRIENE C_4 AND CHINESE HAMSTER LUNG FIBROBLASTS (V79A03 CELLS). 1. CHARACTERIZATION OF BINDING

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Abstract

A novel, specific, and potent biological action of leukotriene C_4 (LTC_4) was demonstrated in the Chinese hamster lung fibroblast cell line V79A03 (V79 cells), namely the conferment of protection against subsequent γ-irradiation. Consequently, studies were conducted to determine whether LTC_4-conferred radioprotection could be attributed to a receptor-mediated phenomenon. Specific binding sites for leukotriene C_4 (LTC_4) were identified and characterized using intact V79 cells incubated at 4°C in the presence of serine-borate, during which time conversion of LTC_4 to LTD_4 or LTE_4 was undetectable. Binding was maximal in a broad region between pH 6.2 and 8.8. Ca^{2+}, Mg^{2+}, and Na^+ were not required for binding, and binding was not altered by GTP, ATP, or cAMP, by leukotrienes B_4, D_4, or E_4, or by the leukotriene end point antagonists LY 171883, FPL 55712, or Revlon 5901-5. Scatchard analyses and kinetic experiments indicated the presence of high-affinity [Kd = 2.5 ± 0.63 nM, approximately 9.9 x 10^5 sites/cell] and low-affinity [Kd = 350 ± 211 nM, approximately 2.7 x 10^6 sites/cell] binding sites. The observed binding characteristics of LTC_4 to V79 cells are consistent with a receptor-mediated phenomenon. In a companion communication which follows this report, we report the subcellular distribution of LTC_4 binding to V79 cells and demonstrate that this binding is unlikely to be attributed principally to interaction with glutathione-S-transferase.

Introduction

Eicosanoids have come under increasing scrutiny as possible agents to alleviate the damaging effects of ionizing radiation and because elevated eicosanoid synthesis by certain tumors is associated with their resistance to chemotherapy and radiation therapy (1). An *in vitro* model system was recently described (2) to evaluate the radioprotective effects of eicosanoids in the Chinese hamster lung fibroblast cell line V79A03 (V79 cells). In this system, pretreatment of V79 cells with leukotriene C_4 (LTC_4) elevated the cellular rate of postirradiation survival. The radioprotective effects of LTC_4 on V79 cells were attenuated if the cells to be treated were harvested from culture flasks by trypsin preincubation (unpublished data), suggesting that the radioprotective effect of LTC_4 depends on the presence of a proteinaceous factor present on the V79 cell surface.

In addition to its effect in V79 cells, pretreatment with LTC_4 was radioprotective in murine hematopoietic stem cells *in vivo* (3) and

enhanced animal survival following otherwise lethal radiation exposure (4). The mechanism(s) by which LTC_4 induced this protection has not been elucidated. The magnitude of the protection in vivo (3) was greater than the protection observed in vitro (4), indicating that specific cellular activities as well as physiological processes at the tissue and organ level may be important in vivo. The V79 cell provides a controlled model system in which to specifically study the cellular responses to LTC_4 that are important in the radioprotective response. In view of the observation that trypsinization attenuated LTC_4-induced radioprotection in the V79 model, studies were conducted to assess the possibility that LTC_4 receptors could be detected on V79 cells. The properties of LTC_4 interaction with V79 cells are described in this communication. In the companion communication that follows this report (5), we describe additional studies that determine the subcellular localization of LTC_4 binding by V79 cells and demonstrate that this binding site is distinct from that of glutathione S transferase, which is known to bind LTC_4.

Methods

Materials. Leukotrienes were a gift from Dr. J. Rokach (Merck Frosst Canada, Inc., Pointe Claire-Dorval, Quebec). Prostaglandin D_2 was a gift from Dr. D. Morton (Upjohn, Kalamazoo, MI). Other unlabeled eicosanoids were obtained from Cayman Chemical (Ann Arbor, MI). Tritiated eicosanoids and Aquasol-2 were obtained from New England Nuclear (Boston, MA). The leukotriene end point antagonists FPL 55712, LY 171883, and Revlon 5901-5 were gifts from Dr. A. Taub (Fison Corp., Bedford, MA), Mr. W. Fields (Lilly Research Laboratories, Indianapolis, IN), and Dr. T. P. Pruss (Rorer Group, Inc., Tuckahoe, NY), respectively. Nucleotides, trypsin, neuraminidase, hyaluronidase, glutathione-S-transferase, and 1-chlor-2,4-dinitrobenzene were obtained from Sigma (St. Louis, MO); Whatman glass-fiber filters (GF/B) from Fisher Scientific (Pittsburgh, PA); Iscove's Modified Dulbecco's Medium (IMDM), Medium 199 (M199), Hank's buffered salt solution (HBSS) and Ca^{2+},Mg^{2+}-free HBSS from MA Bioproducts (Walkersville, MD); α-minimal essential medium (MEM) with Earl's salts and fetal bovine serum (FBS) from Gibco (Grand Island, NY); and KINETIC-LIGAND program from Elsevier-Biosoft (Cambridge, U.K.).

Cell Culture. Cells were grown either in MEM or IMDM supplemented with 10% FBS and maintained as monolayers in both culture flasks and roller bottles at 37°C under humidified 95% air:5% CO_2. Cells were harvested by exposure for 10 min to 0.002% EDTA in phosphate-buffered saline at pH 7.35. Harvested cells were washed, resuspended in HBSS, and counted using a hemocytometer. All cells used in these experiments were harvested during the log growth phase.

Binding Assays. Binding assays were conducted in a reaction buffer composed of HBSS containing 25 mM HEPES and 10 mM serine-borate. Serine-borate was added to block metabolism of LTC_4 (6). After completion of studies to optimize time, pH, and temperature, incubations were conducted in pH 7.35 reaction buffer at 4°C, in a shaking water bath for 30 min. Assay tubes contained 10^5-10^7 cells, 20-70 fmol [^{3}H]-LTC_4 (specific activity 40 Ci/mmol) and reaction buffer in a total volume of 50-100 μl. Nonspecific binding was assessed by determining the binding of [^{3}H]-LTC_4 in the presence of excess unlabeled LTC_4 (16 μM). All binding assays were conducted in triplicate or quadruplicate.

Binding assays were terminated by dilution with 3 ml of ice-cold HBSS. The cell-bound radioactivity was recovered either by centrifugation at 2000 x g for 5 min to pellet the cells followed by aspiration of the supernatant or by filtration through Whatman GF/B filters on a Yeda filtration manifold. Retentates were solubilized in scintillation fluid (Aquasol-2), and bound radioactivity was assessed on a Tracor Analytic Mark III scintillation counter. The centrifugation and filtration methods yielded similar recoveries of tritiated ligand. Scatchard analyses were performed by incubating aliquots of cells with tritiated ligand and graded levels of unlabeled ligand from 0 to 16 μM/assay tube. The resulting displacement curves were transformed to Scatchard plots using LIGAND. All experiments reported herein were replicated at least once, and results are presented as means ± SEM unless otherwise indicated. Kinetic data were evaluated using KINETIC.

Binding Specificity: Eicosanoids and Leukotriene Antagonists. Specificity of LTC_4 binding was assessed by adding 1 μg of selected eicosanoids (100 μl total volume) to assay tubes. The following eicosanoids were individually tested: LTC_4, LTA_4, LTB_4, LTD_4, LTE_4, PGA_2, PGB_2, PGD_2, PGE_2, $PGF_{2\alpha}$, 5-HETE, and 12-HETE. Additional binding assays were conducted in the presence of varying concentrations of the putative leukotriene receptor antagonists LY 171883, FPL 55712, or REV 5901-5 and using graded concentrations of the isomer 14,15-LTC_4.

Cationic and Nucleotide Requirements. Cells were harvested in Ca^{2+},Mg^{2+}-free HBSS that contained 0.002% EDTA. The pelleted cells were washed twice in Ca^{2+},Mg^{2+}-free HBSS and aliquoted into four groups for binding assays. Group (a) binding assays were conducted in 100 μl of Ca^{2+},Mg^{2+}-free HBSS containing 0.02% EDTA; group (b) contained Ca^{2+},Mg^{2+}-free HBSS; group (c) contained complete HBSS (9.5×10^{-4} M Ca^{2+} and 9.0×10^{-4} M Mg^{2+}); and group (d) contained M199. Nucleotide requirements were assessed by modifying the standard ligand assay to include 1 μg/tube of cAMP, ATP, or GTP.

Examination of Other Eicosanoid Receptors on V79 Cells. Specific binding to V79 cells by PGD_2, PGE_2, $PGF_{2\alpha}$, LTD_4, LTE_4, and LTB_4 was examined by a modification of the standard LTC_4 ligand assay replacing the [^{3}H]-LTC_4 with the other [^{3}H]-labeled eicosanoids. Nonspecific binding was determined by adding a 100-fold molar excess of the respective unlabeled eicosanoid.

High-Performance Liquid Chromatography. Purity of leukotrienes was routinely monitored by high-performance liquid chromatography (HPLC) prior to use in binding assays. Reversed-phase HPLC was conducted as previously described (2,7) using either a Beckman or LKB GTi system with an Ultrasphere (Beckman Inc., Columbia, MD) C-18 column, 4.6 x 250 mm, packed with 5-μm particles. Leukotrienes were eluted using 35% (v/v) acetonitrile/water containing 0.1% acetic acid at a pH adjusted to 5.8 with ammonium hydroxide and at a flow rate of 1.0 ml/min. Unlabeled leukotrienes were detected by absorbance at 280 nm. Tritiated leukotrienes were located on chromatographs by collecting fractions and assessing radioactivity with liquid scintillation counting or with an HPLC flow-through radiation monitor (RAMONA-D; IN/US, Inc.).

Metabolism of [^{3}H]-LTC_4 was assessed by incubating 10^6 cells with 112 fmol

[^{3}H]-LTC_4 at 4°C for periods of up to 1 hr. Following incubation, labeled material was extracted from the assay mixture containing cells and medium as described (2). Briefly, the pH of the medium was adjusted to 3.0 with HCl, followed by addition of 100 µl of isopropyl alcohol and 2.0 ml of ethyl acetate. This mixture was sonicated to maximize dissolution of the cellular membrane and centrifuged at 1000 x g for 5 min. More than 98% of the radiolabel was extracted. The extract was evaporated to dryness, resuspended in HPLC mobile phase buffer, and analyzed by HPLC. Radioactivity in the eluate was identified by coelution with known standards.

Statistical Analyses. All experiments were replicated at least once, with equivalent results. Scatchard analyses were derived from displacement curves in duplicate. Other determinations were conducted in triplicate. Differences between treatments were assessed by student's t-test.

Results

Optimization of Binding Assay. The pH dependence of [^{3}H]-LTC_4 binding was examined using whole cells. Cells were pelleted by centrifugation, and then resuspended in medium in which the pH of the reaction buffer was adjusted with NaOH or HCl to 6.2, 6.8, 7.35, 8.0, 8.4, 8.8, or 9.2. Binding assays were conducted as described in Methods. Binding plateaued between pH 6.2 and 8.0 (Fig. 1) and decreased at higher pH values (>8.0). Subsequent binding assays were conducted at pH 7.35.

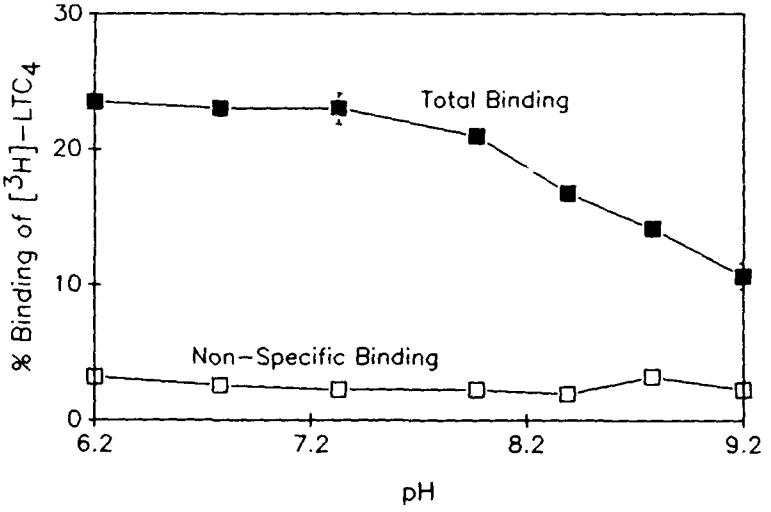


Fig. 1. Optimum pH for Binding of [^{3}H]-LTC_4 to V79 Cells. Total binding (■) was determined in assay tubes containing 10^6 cells and 1.3 nM [^{3}H]-LTC_4, incubated in 50 µl assay buffer in which pH was adjusted with either NaOH or HCl. Nonspecific binding (□) was assessed in parallel tubes containing 16 µM unlabeled LTC_4. Mean ± S.D.

Binding assays were optimized for incubation time and temperature. Parallel sets of tubes with or without 1 μM unlabeled LTC_4 were incubated for 0, 15, 30, 45, or 60 min at 4°, 22° and 37°C. This experiment was conducted in the absence of serine-borate to assess the contribution of cellular levels of γ-glutamyl transpeptidase to binding. Radiolabeled LTC_4 was rapidly bound by cells (Fig. 2) and binding approached maximal levels within 15 min at either 4°C or 22°C. Binding at 37°C was lower and more variable than at lower temperatures (data not shown). HPLC analyses (reported in reference 2) confirmed catabolism of [^{3}H]-LTC_4 at the higher temperatures. Parallel groups of tubes were incubated in the absence of cells (cell blanks) at both 4°C and 22°C. Because binding at 4°C and 22°C in both the nonspecific and cell blank groups were similar, for clarity only the data from the 4°C incubation for these two groups are included in Fig. 2. A better estimate of nonspecific binding was obtained by increasing unlabeled LTC_4 from 1 μM to 16 μM. This reduced nonspecifically bound radioactivity to levels not different from bound radioactivity in the absence of cells (data not shown). On the basis of these results, subsequent binding assays were conducted in triplicate at 4°C for 30 min, and nonspecific binding was assessed in the presence of 16 μM LTC_4.

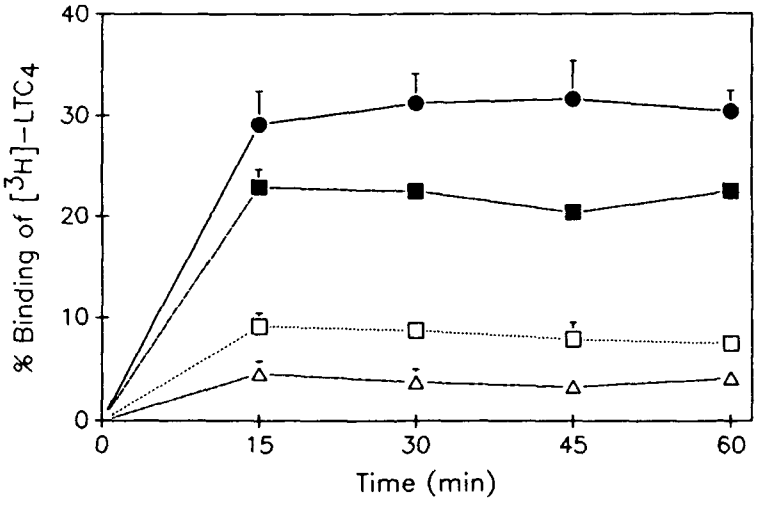
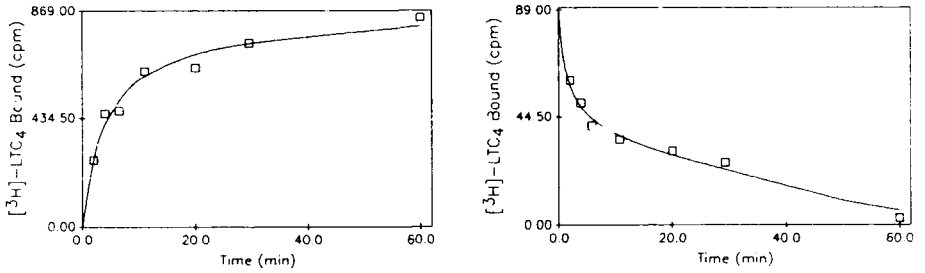


Fig. 2. Time and Temperature Dependence of [^{3}H]-LTC_4 Binding to V79 Cells. Assay tubes containing 5 x 10^5 cells and 1.3 nM [^{3}H]-LTC_4 in 50 μl assay buffer were incubated for the indicated times at 4°C (●) or 24°C (■). Parallel series of tubes were incubated at 4°C containing 1 μM unlabeled LTC_4 (□) or lacking cells (Δ). Mean ± S.D.

Association and Dissociation of [^{3}H]-LTC_4. To determine the rate of association of [^{3}H]-LTC_4 with the receptor, aliquots of the cells were incubated for 2, 4, 6, 10, 20, 30, and 60 min (Fig. 3). To determine the dissociation rate, 10 min after the incubations were initiated, 16 μM of unlabeled LTC_4 was added to a parallel set, and those incubations were terminated at the indicated times (Fig. 4). KINETIC was used to generate lines that best fit the data.



Association (**Fig. 3**) and Dissociation (**Fig. 4**) of [^{3}H]-LTC_4 by Intact V79 Cells. 10^6 cells/tube were incubated at 4°C with 1.3 nM [^{3}H]-LTC_4 in 50 µl assay buffer. Dissociation rate was monitored in tubes to which 10 µl unlabeled LTC_4 (16 µM final concentration) was added after an initial incubation of 10 min. All incubations were terminated by filtration at indicated times. Curves were generated using KINETIC and a two-site model (Association: 54% K_{ass1} = 0.032 ± 0.028; 46% K_{ass2} = 0.86 ± 0.23. Dissociation: 41% K_{dss1} = 0.032 ± 0.013; 59% K_{dss2} = 0.77 ± 1.25).

Identification of Binding Products. V79 cells metabolize LTC_4 to LTD_4 and LTE_4 when incubations are conducted at 37°C (2). Therefore, reversed phase HPLC was used to assess radiolabeled products obtained after incubation of V79 cells with [^{3}H]-LTC_4. No metabolism of LTC_4 was detected during incubations at 4°C for up to 1 hr (Fig. 5), demonstrating that binding was attributable to LTC_4 and not a metabolic product such as LTD_4 or LTE_4. This also suggested that the binding process did not alter the ligand, and that when released from the binding site LTC_4 could bind again.

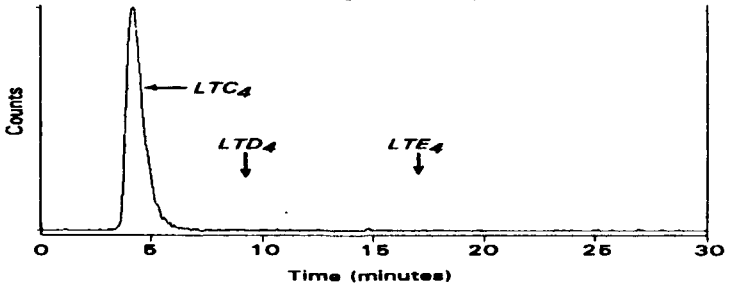


Fig. 5. High Performance Liquid Chromatrography (HPLC) Analysis of [^{3}H]-LTC_4 Binding Products. V79 cells were incubated with [^{3}H]-LTC_4, then extracts were analyzed by HPLC as described in Methods. Units of radioactivity are not provided on the abscissa, because area under the LTC_4 peak (10,000 dpm) accounts for all labeled material recovered. Arrows indicate regions of elution of authentic standards of LTD_4 and LTE_4.

Binding Specificity. V79 cells were incubated with selected tritiated eicosanoids to determine the presence of other classes of eicosanoid receptors or specific binding sites. Incubations with tritiated PGD_2, PGE_2, and $PGF_{2\alpha}$ did not result in tracer binding that was displaceable by 100-fold molar excess of the respective unlabeled ligand. Incubations

with tritiated LTD_4 resulted in minimal and inconsistent levels of specific binding (data not shown). Glutathione at concentrations of up to 3 μM had no effect on [^{3}H]-LTC_4 binding (data not shown).

Table 1. Specificity of [^{3}H]-LTC_4 Binding.[a]

Eicosanoid	Control Binding (%)[b]
LTC_4	16.6 ± 1.9[c]
LTA_4	98.4 ± 5.2
LTB_4	105.4 ± 8.5
LTD_4	107.3 ± 15.9
LTE_4	94.5 ± 8.7
PGA_2	79.8 ± 8.5[c]
PGB_2	107.5 ± 5.1
PGD_2	95.6 ± 3.7
PGE_2	106.9 ± 3.5
$PGF_{2\alpha}$	126.3 ± 11.9
5-HETE	100.1 ± 4.8
12-HETE	116.3 ± 13.6

[a] 10^6 cells/tube in 50 μl buffer were incubated at 4°C for 30 min with 30 fmol [^{3}H]-LTC_4 and 1 μg of each eicosanoid.
[b] ($\bar{x}$ ± SEM)
[c] $P<.001$ by t-test

Table 1 shows the capacity of various eicosanoids to displace [^{3}H]-LTC_4. Only LTC_4 and PGA_2 competed with [^{3}H]-LTC_4 for binding to V79 cells. The isomer of LTC_4 14,15-LTC_4 competed with [^{3}H]-LTC_4 for binding, although the displacement curve for 14,15-LTC_4 was displaced appreciably to the right of that by LTC_4 (Fig. 6).

To assess further the specificity of LTC_4 binding, V79 cells were incubated with compounds that have reported leukotriene antagonist activity. The compounds LY 171883, FPL 55712, or REV 5901-5 did not affect LTC_4 binding (Fig. 7). Interestingly, binding actually tended to be higher in the presence of LY171883 or REV5901-5.

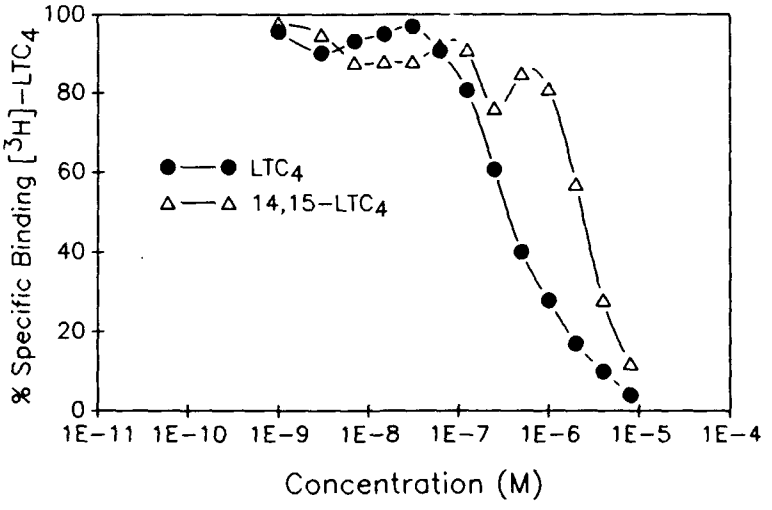


Fig. 6. Competition by 14,15-LTC_4 for Binding to V79 Cells. Parallel displacement curves were conducted using LTC_4 (•) or the 14,15 isomer of LTC_4 (Δ).

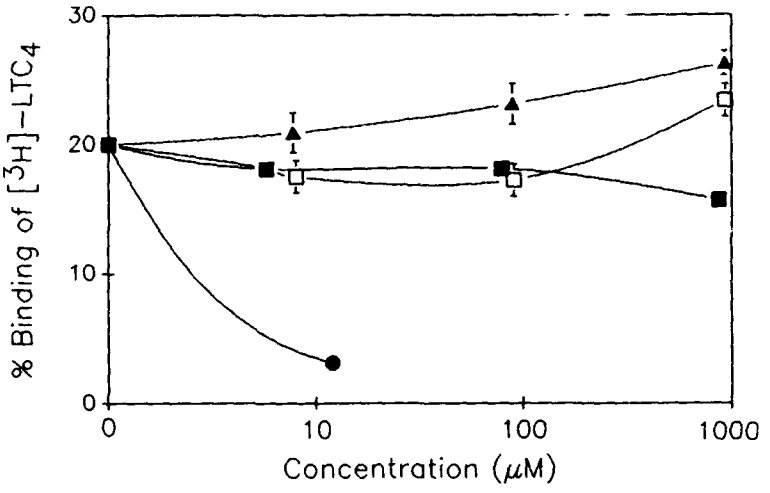


Fig. 7. Effect of Leukotriene Antagonists on LTC_4 Binding. 10^6 V79 cells were incubated at 4°C for 30 min with 1 nM [^{3}H]-LTC_4 and differing concentrations of either LTC_4 (•), LY 171883 (□), FPL 55712 (▪), or REV 5901-5 ( ).

LTC_4 and LTD_4 binding to tissues may be influenced by monovalent and divalent cations (8,9,10), and nucleotides (10,11). To determine if media components influence the binding of LTC_4, V79 cells were incubated with [^{3}H]-LTC_4 in media of differing compositions. No difference in binding was detected when assays were conducted in M199, HBSS, Ca^{2+}/Mg^{2+}-free HBSS, Ca^{2+}/Mg^{2+}-free HBSS containing 0.01% EDTA, or 25 mM Tris-1 mM $CaCl_2$ (pH 7.35 in all buffers). Also, binding of [^{3}H]-LTC_4 was not significantly altered by inclusion of 1 μg/100 μl of cAMP, ATP, or GTP in HBSS (data not shown).

Scatchard Analyses. Scatchard analyses were conducted using either live cells or cells that were previously frozen and stored in pellets at -80°C. To determine if freezing affected Scatchard analyses, pools of V79 cells were divided into two aliquots. One aliquot was placed on ice for 12 hr, while the other was pelleted, frozen, and stored for 12 hr at -80°C. Subsequent Scatchard analyses revealed no differences in LTC_4 binding abundance or affinities (data not shown). A summary of Scatchard analyses conducted on eight separate pools of cells was curvilinear, having high-affinity [Kd = 2.5 ± 0.63 nM; 9.9 x 10^5 sites/cell) and low-affinity [Kd = 350 ± 211 nM; 2.7 x 10^6 sites/cell] binding sites (Fig. 8).

Discussion

LTC_4 plays a role in controlling the lungs (12,13,14,15), cardiovascular system (12,16) and smooth muscle (12). LTC_4-immunoreactive neurons identified in the hypothalamic median eminence (17), in which LTC_4 has been demonstrated as a potent mediator of gonadotropin-releasing hormone (18) and LTC_4 in very small amounts induces gonadotropin release from anterior pituitary cells in vitro (17). Binding of LTC_4 to rat lung fibroblasts was

correlated with stimulation of collagen by the treated cells (19). However, the contribution of LTC_4 in regulation of some physiologic processes has proven controversial because a number of cell and tissue types contain "binding sites" for LTC_4 without demonstrable biologic responsiveness to LTC_4 (20).

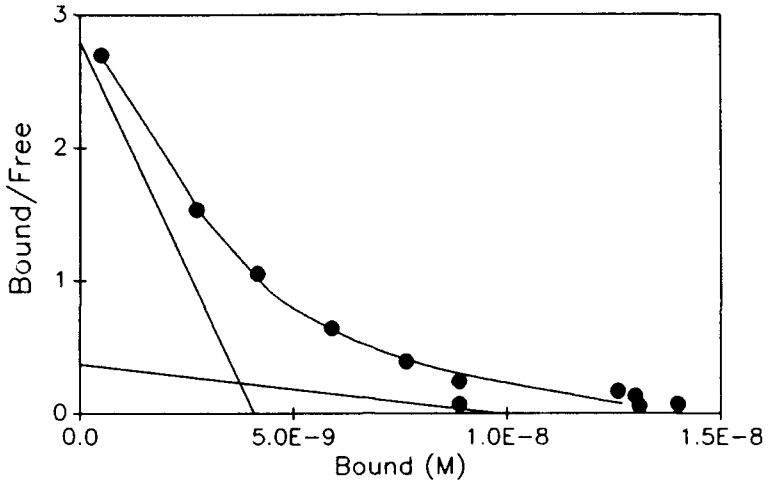


Fig. 8. Scatchard Analysis of $[^3H]$-LTC_4 Binding Sites on V79 Cells. Incubations were conducted at 4°C for 30 min using 10^6 cells and 1.3 nM of $[^3H]$-LTC_4 per tube, while concentrations of unlabeled LTC_4 ranged from 0-16 μM. Displacement curves were transformed to Scatchard plots using LIGAND, which revealed high-affinity (Kd = 2.5 ± 0.63 nM) and low-affinity (Kd = 350 ± 211 nM) binding sites.

A series of criteria has been established for distinguishing receptor-mediated events from events of a less specific nature. One summarization of the events that classically elevate the events of a tissue-ligand interaction to a receptor-mediated event (21) included the criteria of drug displacement, correlation between drug affinity in vitro and pharmacological potency, regional distribution, subcellular distribution, stereospecificity, saturability, reversibility and high affinity. Our studies indicate that the interaction of LTC_4 with V79 cells satisfies most, but not all, of the criteria describing a classical "receptor": our studies demonstrate reasonable levels of saturability, reversibility, stereospecificity, pharmacologic potency, and high affinity. Regional distribution of binding is not applicable in our studies using a cell line. The issue of subcellular distribution is assessed in the companion communication (5), and the issue of drug displacement is addressed below.

As outlined in Table 1, competition with $[^3H]$-LTC_4 for binding to the intact V79 cell was specific for LTC_4 and was not displaceable to a significant degree by other classes of leukotrienes or by a molecular component of LTC_4, glutathione. The 14,15-isomer of LTC_4 exhibited approximately 10% potency. LTC_4 binding was not displaceable by a number of structurally similar and pharmacologically similar compounds, including

a long list of prostaglandins (Table 1). The inhibition of binding by PGA_2 was unusual but repeatable and it is not known whether this was a direct or indirect effect on the receptor protein. Specific antagonists for LTC_4 are not presently available. There are several antagonists for LTD_4 and LTE_4, including FPL 77512 (9,22), LY 171883 (23), and REV 5901-5 (24), but these compounds do not antagonize LTC_4 effects or binding to a great degree (9,22,24). Therefore, it was not surprising that they were ineffective antagonists to LTC_4 binding by the V79 cell, but they indicate common specificity patterns among LTC_4 receptors. The specificity of the receptor was further confirmed when these LTD_4 and LTE_4 antagonists did not compete for the LTC_4 binding sites.

The curvilinear Scatchard plots describing LTC_4 binding to intact V79 cells suggested either multiple classes of binding sites or negative cooperativity in LTC_4 binding. Because the curvilinear Scatchard plots were not demonstrable in binding assays using plasma membrane or nuclear V79 cell preparations (5), it seems most likely that multiple classes of binding sites were revealed in binding assays using intact V79 cells. While the low-affinity binding sites on V79 cells were more abundant than the high-affinity sites (low-affinity binding was approximately 70% of total specific binding), the high-affinity sites on V79 cells were relatively abundant in comparison with reported abundance in other cell lines. For example, the cumulative binding site abundance using intact V79 cells was approximately tenfold higher than that reported for the DDT1M2F hamster smooth muscle cell line (25).

The binding affinities of LTC_4 to V79 cells correlated with doses of LTC_4 that decreased the radiosensitivity of this cell line. In radioprotection studies, pretreatment with 0.5×10^{-6} M LTC_4 was minimally effective, while 2.5×10^{-6} M LTC_4 conferred greater radioprotection (2). Thus LTC_4-conferred radioprotection in the V79 cell line, while not a usual physiologic event, nevertheless provides a correlation of pharmacological potency with the affinity range determined for LTC_4 binding.

V79 cells metabolize LTC_4 to LTD_4 and LTE_4 when cultured at 37°C, and both cells and tissue culture medium possess γ-glutamyl transpeptidase activity (2). Enhanced degradation of LTC_4 may explain the decrease in binding observed with increasing incubation temperature (Fig. 2). The addition of serine-borate, a transition state inhibitor of γ-glutamyl transpeptidase (6), decreased the possibility of LTC_4 degradation in later experiments. HPLC analysis of products from the binding assay suggested that LTC_4 was not decomposed or metabolized during binding studies conducted at 4°C.

The LTC_4 binding site in V79 cells, like the rat glomeruli binding site (26), did not appear to require Na^+, Ca^{2+}, or Mg^{2+} for binding activity. Guanine nucleotides were reported to regulate the binding of LTD_4 to guinea pig lung membranes (10), but appeared to have no effect on LTC_4 receptors in either the guinea pig myocardium (8) or in V79 cells. The V79 cell was reportedly responsive to agents that stimulate cyclic AMP, including prostaglandins (27). The latter is interesting in light of our inability to demonstrate specific binding of several tritiated prostaglandins by this cell line. In particular, we did not observe specific binding of PGE_2 by V79 cells. It was reported in a prior study that pretreatment with PGE_2 did not confer radioprotection of V79 cells (28), which is not

inconsistent with the hypothesis that eicosanoid-induced radioprotection is receptor mediated.

The mechanism by which LTC_4 confers radioprotection to the V79 cell is unknown, although leukotrienes may induce synthesis of cAMP (29), cGMP (29), or other eicosanoids (30), which may in turn have protective effects. Further studies are needed to clarify the mechanism by which LTC_4 protects V79 cells from the deleterious effects of irradiation, although our studies suggest that LTC_4 may interact with V79 cells through a receptor-mediated event. In view of its abundant LTC_4 binding capacity, the V79 cell line may provide both an excellent source for leukotriene receptor purification and a model for defining certain cellular responses to leukotrienes.

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